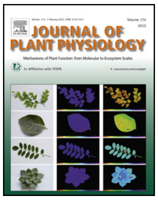
Journal of Plant Physiology 174 (2015) 16–25

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph



Physiology

Balanced allocation of organic acids and biomass for phosphorus and nitrogen demand in the fynbos legume *Podalyria calyptrata*

Pravin M. Maistry[a], A. Muthama Muasya[a], Alex J. Valentine[b], Samson B.M. Chimphango[a,*]

[a] *Department of Biological Sciences, University of Cape Town, Private Bag X3, Rondebosch 7701, South Africa*
[b] *Department of Botany and Zoology, University of Stellenbosch, Private Bag X1, Matieland 7602, South Africa*

ARTICLE INFO

Article history:
Received 26 August 2014
Received in revised form 8 October 2014
Accepted 10 October 2014
Available online 23 October 2014

Keywords:
N × P interaction
N_2-fixation
P-acquisition
P-limitation
Phosphoenolpyruvate carboxylase

ABSTRACT

Podalyria calyptrata is from fynbos soils with low availability of phosphorus (P) and nitrogen (N). We investigated the physiological basis for tolerance of low P supply in nodulated *P. calyptrata* and examined responses to increased supply of combined-N as $Ca(NO_3)_2$ and P. It was hypothesized that increasing supply of combined-N would stimulate P-acquisition mechanisms and enhance plant growth with high P supply. Biomass, leaf [N] and [P], organic acid and phosphatase root exudates, and phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH) activity in nodules and roots were examined in two N × P experiments. Low P supply decreased leaf [P] and limited growth, decreasing the nodule:root ratio but increasing nodular PEPC and MDH activity for enhanced P-acquisition or P-utilization. At low P supply, a N-induced demand for P increased root exudation of citrate and PEPC and MDH activity in roots. Greater combined-N supply inhibited nodulation more at low P supply than at high P supply. With a P-induced demand for N the plants nodulated prolifically and increased combined-N supply did not enhance plant growth. The physiological basis for N_2-fixing *P. calyptrata* tolerating growth at low P supply and responding to greater P supply is through balanced acquisition of P and N for plant demand.

Introduction

The Core Cape Subregion (CCR) is a Mediterranean-type ecosystem dominated by sclerophyllous fynbos vegetation (Manning and Goldblatt, 2012) found on oligotrophic soils of the Table Mountain Sandstone Group with low availability of Bray No. 2 phosphorus (P) of about 4 mg P kg^{-1} and total nitrogen (N) of 1–2 g N kg^{-1} (Mitchell et al., 1984; Stock and Lewis, 1986). The shrub legume *Podalyria calyptrata* (Retz.) Willd. (Fabaceae, Tribe Podalyrieae) is found in the south-west CCR especially in mountain fynbos on the Cape Peninsula (Schutte-Vlok and van Wyk, 2011). The species is favoured for horticulture with its attractive silver-green foliage and fragrant purple-white blossoms that attract bees, butterflies and birds. In a glasshouse study (Maistry et al., 2013), *P. calyptrata* showed superior nodulation at low P supply compared with 16 other fynbos legumes, and also demonstrated traits such as high P-use efficiency, poor down-regulation of P-uptake, storage of P in the shoot and a high seed P content. These traits are typical of plants from oligotrophic soils (Hawkins et al., 2007; Ostertag, 2010; Lambers et al., 2011). The physiological basis for *P. calyptrata* to tolerate low availability of P is yet to be reported. Increased exudation of organic acids and phosphatase activity by roots may enhance acquisition of P from soils with low availability of P (Lambers et al., 2006). The exudation of organic acids by roots was shown to increase with limited P supply (Pearse et al., 2006; Pang et al., 2010) and may enhance the availability of P in the rhizosphere by chelating cations that precipitate P, or through ligand exchange by displacing already sorbed P (Hinsinger, 2001; Ryan et al., 2001). In addition, soil organic matter accounts for 60–80% of P in fynbos soils (Mitchell et al., 1984; Witkowski and Mitchell, 1987), and root-associated acid phosphatases may further enhance the availability of P for plants by hydrolyzing these organic sources of P (Tarafdar and Claassen, 1988; Gilbert et al., 1999).

The addition of N to soils with low availability of P was also observed to increase the extracellular phosphatase activity of roots and soil (Olander and Vitousek, 2000; Treseder and Vitousek, 2001). It is possible that the induction of the P-acquisition mechanism by greater supply of combined-N may be in response to lower availability of P relative to N, i.e., a greater demand for P induced by N (Phoenix et al., 2003). Colimitation of the growth of legume plants by N and P (Power et al., 2010; Maistry et al., 2013), implies that plant responses to N and P will depend on the relative supply levels

---

* Corresponding author. Tel.: +27 21 650 2964; fax: +27 21 650 4041.
E-mail address: samson.chimphango@uct.ac.za (S.B.M. Chimphango).

http://dx.doi.org/10.1016/j.jplph.2014.10.005
0176-1617/

of the two nutrients, so that the interactive effects of N and P supply on the mechanisms for acquisition of P and N and growth need to be closely examined. Plants can regulate their N:P ratios relative to that of the supply N:P ratio (Güsewell, 2004; Elser et al., 2010). Thus, with increasing supply of combined-N under P-limiting conditions legume plants would need to allocate resources away from the acquisition of N towards acquisition of the more limiting P (Bloom et al., 1985) through decreasing nodulation (Streeter, 1988) but increasing phosphatase activity or release of organic acids. On the other hand, stimulation of growth in the fynbos legume *Cyclopia genistoides* (L.) R.Br. by adding P, was observed to decrease biomass allocation to roots for reduced investment in acquisition of P, but induce nodulation so as to enhance N supply to meet the P-induced demand for N (Maistry et al., 2013). These physiological adjustments for acquisition of P and N would be to bring the supply N:P ratio closer to the demand N:P ratio for growth (Bloom et al., 1985; Houlton et al., 2008; Vitousek et al., 2010).

Consistent with the resource allocation theory for balanced acquisition of P and N (Bloom et al., 1985), decreased nodulation with low P supply has been attributed to reduced demand for N due to limitation of growth by P-deficiency (Almeida et al., 2000; Sulieman et al., 2013), often accompanied by greater investment of resources for P-use (Theodorou and Plaxton, 1993; Araujo et al., 2008) or P-acquisition such as greater root biomass, phosphatase activity and exudation of organic acids (Lambers et al., 2006). At the biochemical level, increased phosphoenolpyruvate carboxylase (PEPC) activity at low P supply may enhance P-acquisition through greater synthesis of citrate and malate as in cluster roots of *Lupinus albus* L. (Johnson et al., 1996; Neumann and Römheld, 1999), or recycle inorganic phosphate (P_i) by liberating P_i from phosphoenolpyruvate (PEP) when catalyzing its carboxylation to oxaloacetate (OAA) (Theodorou and Plaxton, 1993). In addition, carbon (C) from the sequential action of increased PEPC, malate dehydrogenase (MDH) and malic enzyme (ME) activity can facilitate continued mitochondrial respiration in the tricarboxylic acid (TCA) cycle with limited demand for P, by bypassing the adenylate requiring production of pyruvate through pyruvate kinase (PK) (Duff et al., 1989; Theodorou and Plaxton, 1993). In N_2-fixing plants receiving adequate P supply, PEPC also provides C for replenishing TCA cycle intermediates such as citrate, used in the assimilation of N (Cramer et al., 1993) or in root exudates (Johnson et al., 1994). Furthermore, OAA that is derived from PEPC may be transaminated to aspartate via aspartate aminotransferase (AAT) for N-assimilation (Coker and Schubert, 1981), or converted by MDH to malate for respiring bacteroids (Vance et al., 1985; Rosendahl et al., 1990; Fischinger and Schulze, 2010). Therefore PEPC, MDH and AAT play a critical role in coordinating the flow of C between N and P pools in nodules (Vance and Heichel, 1991; Smith et al., 2000; Colebatch et al., 2004; Fischinger and Schulze, 2010).

Inhibition of nitrogenase activity by factors such as low P supply (Sa and Israel, 1991) or O_2 (Laing et al., 1979), reduces synthesis and concentration of organic acids in nodules (Rosendahl et al., 1990; Vance and Heichel, 1991) possibly due to low demand for malate by bacteroids or for C skeletons in the assimilation of N. Given that tissue [P] and [N] correlate positively (Garten, 1976) and that low P supply decreased nodulation in several studies (Olivera et al., 2004; Araujo et al., 2008; Maistry et al., 2013), P-limited N_2-fixing plants have, therefore, low demand for N (Almeida et al., 2000; Sulieman et al., 2013). Thus, the enhanced expression of PEPC and MDH genes as observed in nodules and cluster roots of *L. albus* at low P supply (Uhde-Stone et al., 2003) most likely is for allocation of C for acquisition or recycling of P. Alternatively, low demand for N at low P supply may not be responsible for reduced allocation of resources to N_2-fixation. Responses to meet the greater demand for P instead may negatively affect N_2-fixation and assimilation of the fixed N_2, because the diversion of up to 25% of plant C for exudation of citrate and malate (Dinkelaker et al., 1989; Lambers et al., 2006) could compete with energy supply for N_2-fixation and plant growth. It has recently been reported that the diversion of C for enhanced synthesis of organic acids in *Lupinus angustifolius* L. may compete with energy supply for N_2-fixation and N-assimilation (Le Roux et al., 2008).

We investigated the physiological basis for tolerance of limiting P supply in nodulated *P. calyptrata* and examined responses to increased supply of combined-N and P. This was achieved by studying growth responses and the mechanisms for acquisition of P and N and assimilation of N at low and high P supply while increasing the supply of combined-N. It was anticipated that low P supply would limit plant growth and increase mechanisms for P-acquisition. It was hypothesized that increased supply of combined-N would also stimulate P-acquisition mechanisms associated with greater P-demand and result in enhanced plant growth with high P supply. The responses of plant biomass, leaf [N] and [P] and leaf N:P ratio, extracellular phosphatase activity and organic acid exudates of roots, and PEPC, PK, MDH, ME, and AAT activity in nodules and roots to N and P supply were examined in two N × P experiments.

Materials and methods

Growth conditions for sand culture

Seeds of *Podalyria calyptrata* (1.75 ± 0.54 mg N seed^{-1}, 0.149 ± 0.003 mg P seed^{-1}) were obtained from a natural population growing in Table Mountain National Park. The seeds were soaked overnight in boiled water then sown in seedling trays containing acid-washed sand. Immediately after emergence, each seedling was inoculated with rhizobia isolated from nodules harvested from plants of the same population in Table Mountain National Park and inoculum prepared according to Vincent (1970). The rhizobia isolated from nodules were fast growing and creamy-white to watery in appearance (personal observation), typical of the *Burkholderia tuberum* strain that nodulates *P. calyptrata* (Sprent et al., 2013). Four weeks after emergence, seedlings of similar size were transplanted into plastic pots filled with 3 kg of acid-washed sand and inoculated with rhizobia. Seedlings were thinned to one per pot after a further four weeks, and there were five pots as replicates for each of the eight treatments ($n = 5$). The concentration of $Ca(NO_3)_2$ and KH_2PO_4 in the solution was adjusted to supply the appropriate nitrate concentrations of 100, 300, 500 and 700 μM and P concentrations of 10 μM and 100 μM, respectively. Therefore with increasing provision of combined-N the experimentally administered N:P supply ratio (by mass) at low P was 9, 27, 45 and 63, and at high P supply the ratio was 1, 3, 5 and 6. To compensate for the adjustments in $Ca(NO_3)_2$, $CaSO_4$ was added to the nutrient solution so that all plants received 200 μM Ca. In addition to $Ca(NO_3)_2$, KH_2PO_4 and $CaSO_4$, the complete nutrient solution also contained (μM): (200) K_2SO_4, (54) $MgSO_4$, (0.24) $MnSO_4$, (0.10) $ZnSO_4$, (0.02) $CuSO_4$, (2.4) H_3BO_3, (0.03) Na_2MoO_4, and (10) Fe-EDTA at pH 6.2, as used in the culture of legumes and Proteaceae from the CCR (Power et al., 2010; Maistry et al., 2013) and south-west Australia (Shane et al., 2004). The plants were watered three times a week with 200 mL of nutrient solution. Pots were flushed with 1 L tap water once a week to prevent salt accumulation. Plants were grown for 21 weeks from April 2011 to August 2011 in a glasshouse at the University of Cape Town (UCT) (S 33° 57.353′; E 18° 27.742′) with an average daytime temperature of 21 °C controlled in the range of 20–30 °C inside the glasshouse. Pots were placed on trolleys that were rearranged weekly.